Variable lipemic response to dietary soy protein in healthy, normolipemic men

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ABSTRACT We found previously that dietary soy protein, compared with casein, reduced plasma LDL cholesterol and increased HDL cholesterol concentrations in healthy women and men. However, there was considerable variation among individuals. The aim of this study was to characterize the lipoprotein responsiveness of individuals to examine whether different response patterns could be identified. Nine normolipemic men consumed 2 liquid-formula diets of identical composition except that the protein component was either soy protein or casein. After 1 mo of consuming each diet, the subjects’ plasma HDL cholesterol (P < 0.01) and apolipoprotein (apo) A-I (P < 0.05) concentrations were increased by the soy-protein diet whereas the ratio of LDL cholesterol to HDL cholesterol was decreased (P < 0.01); total cholesterol, triacylglycerol, LDL cholesterol, apo B and apo A-II were insignificantly affected. In 5 individuals, however, soy protein reduced mean LDL cholesterol, LDL₂ cholesterol, and LDL₃ apo B concentrations by 26% and plasma apo B by 16%, whereas HDL cholesterol increased by 11%. In 3 other individuals, soy protein increased mean HDL cholesterol by 17% and plasma apo A-I by 12%, but did not lower LDL. In 1 subject, soy protein decreased LDL₂ cholesterol by 11% and increased plasma triacylglycerol by 40%, but neither HDL cholesterol nor apo A-I increased. We identified 3 types of lipemic response to dietary soy protein involving a reduction in atherogenic LDL and increase in antiatherogenic HDL. In most subjects, the effects on both LDL and HDL were favorable, although fewer experienced either an increase in HDL or a decrease in LDL₂.

KEY WORDS Soy protein, casein, formula diets, lipoproteins, lipids, apolipoprotein A-I, apolipoprotein A-II, apolipoprotein B, HDL cholesterol, LDL cholesterol, men

INTRODUCTION

The low-fat diets recommended by the National Cholesterol Education Program (NCEP) (1) are designed to lower LDL cholesterol (LDL-C) concentrations and thus reduce the risk of new and recurrent cardiovascular disease. The preventive effects of such diets may, however, be less than expected. Apart from inadequate compliance, lack of a sufficient effect can in some individuals be due to unresponsiveness to the dietary changes (2–4). A further problem may be that the cholesterol-lowering diets tend to reduce HDL cholesterol (HDL-C), increase triacylglycerol concentrations (3, 5), and shift the LDL particle size distribution from larger and buoyant to smaller and dense (5), all of which may diminish the beneficial effect of the LDL reduction. A solution to some of those problems might be the incorporation of dietary components with beneficial lipid effects of their own into low-fat diets; such a combination of several dietary modifications may achieve a more comprehensive reduction in the lipid risk factors. Because soy protein not only lowers LDL-C, but, in addition, decreases triacylglycerol (6, 7) and increases HDL-C concentrations (8), it might play a useful role in such a combination of dietary changes.

The incorporation of soy protein into cholesterol-lowering diets may thus have the potential of further reducing cardiovascular risk. Before the use of soy protein in such diets can be generally recommended, more studies are required to resolve the following problems:

1) Because the cholesterol-lowering effect of soy protein increases with the amount consumed (6), high intakes will be more effective than low intakes, which raises the question of the long-term safety of high soy-protein intakes (9).

2) A high intake of soy-protein preparations results in a high intake of their nonprotein constituents, such as isoflavones and other compounds, which may have beneficial effects, as suggested by the favorable changes in lipids observed in rhesus monkeys (10), but which also might have adverse effects.

3) In human studies of dietary soy protein there has been a marked variability in the lipemic response, which has not been adequately explained (11).

4) In a previous study (8), LDL-C concentrations did not decrease and HDL-C did not increase in some individuals in response to dietary soy protein as they did in most subjects. The aim of the present study was therefore to examine in detail the individual lipemic response of normolipemic men to dietary soy protein.
SUBJECTS AND METHODS

Subjects

Nine healthy men aged 21–64 y (mean 37 y) were recruited among friends and colleagues of the laboratory staff. All men were normolipemic at initial screening, with mean fasting concentrations of 5.1 mmol total cholesterol/L (range: 3.9–6.7 mmol/L), 0.93 mmol triacylglycerol/L (range: 0.4–2.1 mmol/L), 3.2 mmol LDL-C/L (range: 2.4–4.9 mmol/L), and 1.44 mmol HDL-C/L (range: 0.8–2.1 mmol/L), within the 5th and the 95th percentiles of the adult Danish population. The subjects were nonobese with a body mass index (in kg/m²) between 21.7 and 25.1 (mean: 22.9), had no evidence of abnormal liver, kidney, or thyroid function, and had normal fasting plasma glucose concentrations. The protocol was approved by the regional committee on human experimentation.

Experimental design

The subjects were studied during 3 dietary periods: in the first, most subjects (n = 5) ate their usual, self-selected, solid-food diet; in the second and third, subjects consumed liquid-formula diets of identical composition except for the protein component, which was either casein or soy protein. The liquid formulas were consumed for 33 d (n = 2) or 45 d (n = 7), and the dietary periods were separated by an interval of 53 ± 33 days (x ± SD) in which the subjects ate self-selected, solid-food diets. The subjects alternately started on the casein or the soy-protein diet. Fasting blood samples were drawn throughout the study.

Diets

The liquid-formula diets, described previously in detail (8), contained 20% of energy as protein, 55% as carbohydrate, and 25% as fat. The protein preparations, both ≥90% pure, were calcium caseinate (Casec; Mead Johnson Laboratories, Evansville, IN) and a soy-protein isolate (Supro 660; Protein Technologies International, St Louis). The mean (±SD) daily intake (n = 7) of soy protein was 154 ± 7.9 g and that of casein was 154 ± 33 g. In both diets the carbohydrate was a cornstarch hydrolysate (Maltodextrin 01915; Cerestar, Haubourdin, France) and the fat component was the high-oleate variant of safflower oil (Oleinate 181; Pacific Vegetable Oil Corporation, San Francisco). Slightly more cholesterol was added to the soy-protein diet than to the casein diet to compensate for the small amount of cholesterol in the casein preparation. The cholesterol intake was 56.4 mg/MJ (236 mg/1000 kcal). Calcium lactate was added to the soy-protein diet to compensate for the high calcium content of the casein preparation; to compensate for the lactate, an equivalent amount was added to the casein diet in the form of sodium lactate. With additional supplements of vitamins, other micronutrients, and salts, the diets fulfilled recommended dietary allowances (12).

The subjects were asked to weigh themselves daily and to increase or decrease the intake of formula to maintain their body weight. Overall, they lost (x ± SD) 1.8 ± 1.44 and 2.3 ± 1.41 kg after the soy and casein diets, respectively (P > 0.6). They were allowed energy-free beverages and were specifically asked not to drink alcohol.

Lipids, lipoproteins, and apolipoproteins

Blood samples were drawn after the subjects had been fasting for ≥12 h and had spent ≥15 min in a recumbent position. Blood was collected in tubes containing potassium EDTA and plasma was immediately separated by low-speed centrifugation at 2000 × g at 4°C for 30 min. Plasma lipids and lipoprotein concentrations were measured according to the Lipid Research Clinics protocol (13), with the modification that HDL was isolated for cholesterol measurement after precipitation of apolipoprotein (apo) B–containing lipoproteins with magnesium chloride and dextran sulfate (14). In addition, the apo B–containing lipoproteins VLDL₁ [Svedberg flotation unit (Sf) 60–400] and VLDL₂ (Sf 20–60), intermediate-density lipoprotein (IDL) (Sf 12–20), and LDL₂ (Sf 0–12) were prepared by cumulative density gradient ultracentrifugation (15). Percentage recoveries of lipoprotein cholesterol from the density gradients were determined by comparison with total plasma cholesterol, and correction for losses was done by using the same percentage recovery for all fractions. Correction for losses of LDL₂ apoB were done by using the same correction factor as for LDL₂ cholesterol. Cholesterol and triacylglycerols were analyzed by enzymatic methodology (Boehringer, Mannheim, Germany). Plasma apo A-I and apo B were measured by radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden). The procedure used for apo A-I was a competitive assay in which a radiiodinated apo A-I competes with the A-I in the plasma sample for binding to a monoclonal anti-apo A-I antibody attached to sepharose microbeads; after incubation, centrifugation, and decanting, the radioactivity associated with the pelleted microbeads was inversely proportional to the amount of apo A-I in the sample. The plasma apo B assay used 2 different monoclonal anti-apo B antibodies, 1 as a trapping antibody bound to sepharose microbeads and the other reporting antibody radiiodinated; after incubation the microbeads were pelleted and counted and the amount of radioactivity was directly proportional to the amount of apo B in the plasma sample. Plasma apo A-II was measured by immunoturbidimetry by using an anti-human apo A-II antibody (Boehringer). The concentration of LDL₂-apo B was determined as the isopropanol-precipitated protein, measured as the difference between total protein and protein soluble in the presence of isopropanol (16), analyzed by a modified Lowry procedure (17); the apo B value was subsequently corrected for preparative losses as mentioned above.

Statistical analysis

Comparison of variations among the 3 different diets in all 9 subjects was done by one-way repeated analysis of variance (ANOVA), whereas comparisons of pairs of diets were based on the Bonferroni multiple comparisons test. Comparison of the effects of soy-protein and casein diets in individual subjects and in subgroups of subjects were done by paired comparisons using Student’s t test, with P < 0.05 as an indicator of significant difference (GraphPad INSTAT version 2.0; GraphPad Software, San Diego).

RESULTS

When a change is made from self-selected, solid-food diets to the liquid formulas, it takes ≥2–3 wk before plasma lipoproteins stabilize at concentrations characteristic of the particular diet (8). The lipid, lipoprotein cholesterol, and apolipoprotein concentrations after >30 d of the self-selected and liquid-formula diets are shown in Table 1. Consumption of both liquid diets caused all plasma concentrations to decrease, particularly those of total and LDL-C (P < 0.001), plasma apo B (P < 0.001), HDL-C (P < 0.001), and plasma apo A-I (P < 0.01). Although the ratio of LDL-C to HDL-C remained unaffected by the casein diet, it
TABLE 1
Plasma concentrations of lipids and apolipoproteins of subjects after > 30 days on each diet

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>Percentage difference (Soy−Cas)</th>
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<tbody>
<tr>
<td></td>
<td>Self-selected</td>
<td>Casein</td>
</tr>
</tbody>
</table>
| Cholesterol (mmol/L)
| 4.56 ± 1.15        | 3.50 ± 0.82 | 3.46 ± 0.63 | 0 ± 14    |
| LDL cholesterol (mmol/L)
| 2.62 ± 0.82        | 2.01 ± 0.77 | 1.72 ± 0.57 | −11 ± 23  |
| Apolipoprotein B (g/L)
| 0.80 ± 0.27        | 0.67 ± 0.24 | 0.61 ± 0.20 | −9 ± 11   |
| LDL2 apolipoprotein B (g/L)
| 0.43 ± 0.19        | 0.36 ± 0.17 | 0.30 ± 0.13 | −5 ± 19   |
| HDL cholesterol (mmol/L)
| 1.39 ± 0.20        | 1.08 ± 0.15 | 1.21 ± 0.17 | 11 ± 7    |
| Apolipoprotein A-I (g/L)
| 1.18 ± 0.07        | 0.93 ± 0.09 | 1.03 ± 0.10 | 10 ± 8    |
| Apolipoprotein A-II (g/L)
| 0.35 ± 0.05        | 0.31 ± 0.05 | 0.30 ± 0.04 | −3 ± 6    |
| Triacylglycerol (mmol/L)
| 0.87 ± 0.36        | 0.71 ± 0.22 | 0.77 ± 0.31 | 2 ± 41    |
| VLDL cholesterol (mmol/L)
| 0.67 ± 0.52        | 0.49 ± 0.22 | 0.56 ± 0.27 | 12 ± 26   |
| HDL:LDL cholesterol   | 1.94 ± 0.77 | 1.96 ± 0.99 | 1.49 ± 0.65 | −22 ± 21  

1± SD; n = 9. Mean percentage differences ± SD between the soy protein and casein diets were calculated from the natural logarithms of the plasma concentrations.

2 Significant differences between diets (P < 0.001) were at least partly explained by the difficulty he had consuming the casein diet, which led to a weight loss of 3.5 kg during this period, and to his extraordinarily low LDL-C during the soy-diet period, he experienced no difficulty consuming the diet and did not lose weight.

The third subgroup consisted of one individual who responded differently from all the others by showing neither a decrease in LDL-C nor an increase in HDL-C with the soy diet. In fact, the mean LDL-C (1.09 mmol/L) and HDL-C (1.17 mmol/L) concentrations were identical with the 2 liquid diets. This subject did, however, respond to the soy-protein diet with reductions in both LDL2 cholesterol (11%, P < 0.0001) and LDL2 apo B (9%, P = 0.06); the soy diet also increased total and VLDL triacylglycerols by 39% and 57%, respectively (P < 0.02).

TABLE 2
Plasma concentrations of lipids and apolipoproteins in subjects after > 30 d of consuming the casein and soy-protein diets

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>Percentage difference (%)</th>
</tr>
</thead>
</table>
| Cholesterol (mmol/L)
| 3.95 ± 0.72        | 3.62 ± 0.78 | −10 ± 5     |
| LDL cholesterol (mmol/L)
| 2.38 ± 0.73        | 1.83 ± 0.66 | −26 ± 8     |
| Apolipoprotein B (g/L)
| 0.48 ± 0.14        | 0.40 ± 0.12 | −19 ± 7     |
| LDL2 cholesterol (mmol/L)
| 2.14 ± 0.54        | 1.71 ± 0.67 | −26 ± 1     |
| Apolipoprotein A-I (g/L)
| 0.76 ± 0.26        | 0.65 ± 0.23 | −16 ± 4     |
| Apolipoprotein A-II (g/L)
| 0.43 ± 0.20        | 0.33 ± 0.16 | −26 ± 6     |
| HDL cholesterol (mmol/L)
| 1.06 ± 0.16        | 1.17 ± 0.16 | 10 ± 7      |
| Apolipoprotein A-I (g/L)
| 0.89 ± 0.11        | 0.99 ± 0.11 | 10 ± 9      |
| Apolipoprotein A-II (g/L)
| 0.29 ± 0.02        | 0.28 ± 0.03 | −4 ± 7      |
| Triacylglycerol (mmol/L)
| 0.78 ± 0.25        | 0.87 ± 0.24 | 12 ± 17     |
| VLDL cholesterol (mmol/L)
| 0.47 ± 0.17        | 0.58 ± 0.23 | 19 ± 28     |
| VLDL cholesterol (mmol/L)
| 0.09 ± 0.04        | 0.13 ± 0.06 | 34 ± 46     |
| VLDL cholesterol (mmol/L)
| 0.18 ± 0.06        | 0.20 ± 0.07 | 6 ± 38      

1± SD; n = 5. IDL, intermediate-density lipoprotein.

2 Calculated from the natural logarithms of the plasma concentrations.

3 Significant differences between diets (P < 0.004) were at least partly due to the mode of selection.

4 Significant difference (P < 0.004) in all subjects.

5 Significant difference (P < 0.02) in all subjects.
DISCUSSION

This study showed marked individual differences in the effects of dietary soy protein on the lipid transport system in plasma. Despite the variability in lipemic response among individuals, soy protein generally affected the major lipid risk factors favorably, as indicated by the reduction in the ratio of LDL-C to HDL-C (Table 1), whereas the casein diet had no such effect. In this respect the casein diet resembled the NCEP Step II diet (1), which similarly was without beneficial effect on the ratio of LDL-C to HDL-C (3). The most common effects of soy protein were increases in HDL-C and apo A-I, which occurred in 6 and 7, respectively, of 9 subjects. Almost as common as the increase in atherogenic LDL-C, LDL2-C, and apo B observed in 6 of 9 sub-
jects. Less favorable effects were the increases in plasma triacylglycerol concentrations observed in 4 subjects and an increase of LDL-C in 1 subject, although the latter may well have been due to extraordinary weight loss during the casein diet period rather than to a paradoxic response to soy protein. Whether the decrease in apo A-II, observed in 4 individuals, reduced cardiovascular risk remains uncertain, although studies in transgenic mice suggest that it might (18–20).

Variability in responsiveness to dietary soy protein has so far received scant attention and little is known about its causes. Because more is known about conditions influencing the individual total and LDL-C response to dietary fat and cholesterol, the relevance of those factors to the present observations are examined below. Varying dietary compliance (21), random fluctuations in lipoprotein concentrations in studies using single blood samples to determine dietary effects (22), differences in adiposity among subjects (23), and differences in the composition of the baseline diet (24) all seem irrelevant to the present study for the following reasons: 1) all the men had excellent dietary compliance as indicated by marked lipemic effects when changing from the self-selected to the formula diets, 2) repeated blood samplings eliminated the effects of random fluctuations of plasma concentrations, 3) all subjects were nonobese, and 4) the casein diet, which served as the baseline diet for determination of the effects of dietary soy protein. Of the several hypotheses proposed to explain variations in the lipemic response to NCEP diets (3), others respond to a high intake of cholesterol with an elevated plasma cholesterol concentration, whereas others respond to a low intake of cholesterol with the HDL elevation. Therefore, the above-mentioned conditions affecting individual LDL responsiveness to low-fat diets do not explain much of the variable responsiveness to soy protein in the present study. We therefore tend to believe that the individual responsiveness to soy protein was determined largely by intrinsically and probably genetic factors.

One intrinsic factor that might be important in the heterogeneous response to dietary soy protein is the variability in the lipemic response to dietary cholesterol, because some individuals respond to a high intake of cholesterol with an elevated plasma cholesterol concentration, whereas others respond to a much lesser extent or not at all (24). We found previously that the LDL-lowering and HDL-elevating effects of soy protein depended on a certain cholesterol intake, because diets otherwise identical in composition but virtually free of cholesterol showed no difference in the lipemic effects of soy protein and casein (8, 26). Because marked individual differences exist in sensitivity to dietary cholesterol in terms of total and HDL-C response (24), it appears plausible that differences in sensitivity to dietary cholesterol might explain parts or all of the variability in response to dietary soy protein. Of the several hypotheses proposed to explain the variable individual sensitivity to dietary cholesterol, one is based on the role of apo E and its different isoforms that result from allelic variation of the APOE gene. Although some investigators have found that the different apo E isoforms explain variations in the lipemic response to NCEP diets (3), others have not (2). We examined the APOE genotypes of our subjects (5 had E3/E4, 3 had E3/E3, and 1 had E2/E3) and found no significant difference in the LDL reduction due to the soy-protein diet between the different genotypes by ANOVA.

### TABLE 3

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Casein</th>
<th>Soy protein</th>
<th>Percentage difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-density lipoprotein (LDL-C) (mmol/L)</td>
<td>2.78 ± 0.58</td>
<td>3.25 ± 0.52</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>Low-density lipoprotein (LDL2-C) (mmol/L)</td>
<td>1.45 ± 0.68</td>
<td>1.48 ± 0.53</td>
<td>7 ± 24</td>
</tr>
<tr>
<td>Low-density lipoprotein (LDL3-C) (mmol/L)</td>
<td>0.23 ± 0.07</td>
<td>0.30 ± 0.07</td>
<td>27 ± 21</td>
</tr>
<tr>
<td>Low-density lipoprotein (LDL4-C) (mmol/L)</td>
<td>1.25 ± 0.64</td>
<td>1.39 ± 0.50</td>
<td>16 ± 19</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>0.53 ± 0.18</td>
<td>0.51 ± 0.18</td>
<td>2 ± 9</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/L)</td>
<td>0.25 ± 0.10</td>
<td>0.25 ± 0.10</td>
<td>2 ± 25</td>
</tr>
<tr>
<td>Apolipoprotein A-II (g/L)</td>
<td>0.12 ± 0.14</td>
<td>0.13 ± 0.19</td>
<td>17 ± 22</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>0.56 ± 0.14</td>
<td>0.46 ± 0.17</td>
<td>24 ± 66</td>
</tr>
<tr>
<td>VLDL triacylglycerol (mmol/L)</td>
<td>0.33 ± 0.13</td>
<td>0.26 ± 0.11</td>
<td>24 ± 85</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.33 ± 0.07</td>
<td>0.46 ± 0.16</td>
<td>30 ± 17</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.06 ± 0.03</td>
<td>0.09 ± 0.06</td>
<td>31 ± 26</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.10 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>29 ± 23</td>
</tr>
</tbody>
</table>

*SD; n = 3. ILD, intermediate-density lipoprotein.*

*Significant differences between diets (P = 0.02) were at least partly due to the mode of selection.*

*Significant difference (P < 0.01) in all subjects.*

The degree of LDL reduction with low-fat diets has been found to correlate with the LDL-C concentration at baseline (3). In this study we likewise found a significant correlation between the decrease in LDL-C with the soy-protein diet and the LDL concentration with the casein diet (r = 0.76, P < 0.02), in agreement with previous studies of the effects of soyprotein (6). The change in HDL-C with a low-fat diet was negatively correlated with the HDL concentration with the basal diet (3), but here we found no correlation between the HDL concentration on the casein diet and the increase of HDL on the soy diet. Weight changes have been shown to modify the LDL responsiveness to low-fat diets: weight gain reduced the LDL-lowering effect whereas weight loss magnified it (25). In this study, the overall weight changes were modest and similar with both the casein and soy-protein diets and so presumably did not contribute to the variable response of LDL-C to soy protein. There was one exception, however, because one subject lost 3.5 kg with the casein diet and maintained his body weight with the soy diet. Because of the known effect of weight loss on LDL, we favor the interpretation that the casein diet plus weight loss had a greater LDL-lowering effect than did the soy diet without weight changes, rather than the alternative interpretation that the soy diet in this subject had a paradoxic LDL-elevating effect. Thus, the above-mentioned conditions affecting individual LDL responsiveness to low-fat diets do not explain much of the variable responsiveness to soy protein in the present study. We therefore tend to believe that the individual responsiveness to soy protein was determined largely by intrinsic and probably genetic factors.
The protocol of this study had the advantages of precise control of composition and intake of the liquid-formula diets, a detailed study of the lipemic response of each individual subject, and a homogeneous study population of normolipemic, nonobese men. The study also had the following limitations if the aim was to apply the findings to the general use of soy protein in preventive diets: 1) the small number of subjects and the absence of women made the study population highly selected and not representative of the general population, 2) the duration of the dietary periods may have been too brief to allow for adaptation to the diets that might take place over extended periods of time, 3) the use of liquid formulas containing only one kind of protein together with the total absence of fibers could have modified the lipemic response compared with a solid-food diet, and 4) the cholesterol content of the diet, although not different from that consumed by a considerable portion of the background population, was higher than that recommended for cholesterol-lowering diets.

Despite the limitations of our present knowledge about the usefulness and safety of dietary soy protein in reducing cardiovascular lipid risk factors, the potentially beneficial effects clearly indicate both the need and justification for more clinical and experimental studies.

We thank Hanne Merete Olsen and Ninna Buch Petersen for their expert performance of the preparative and analytic work and the participants for their perseverance. Joergen Hilden, Department of Biostatistics, the Panum Institute, provided valuable advice on the statistical data analysis.

REFERENCES